indirect result of inflammation, but it is also possible that epidermal stem cells can regulate their niche through Wnt expression. In this feedback model, a dearth of epidermal stem cells mimicked by *Wls* deletion would decrease signaling to the IFE niche, which would in turn promote a hyperproliferative epidermal response to repair the defect.

The hair follicle is used to study the periodic and coordinated activation of stem cells. Both the stem cells and their niche integrate signals originating from each other, surrounding follicles, and other cell types in the skin and beyond to orchestrate replacement of the hair coat (Plikus et al., 2011). Millar and colleagues show that when released from blockade of Wnt signaling, hair follicles rapidly reenter the growth phase. This novel approach to freezing the stem cells and their niche in an arrested, early activation state may prove helpful in the effort to identify other signals that regulate regeneration in this system.

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# Who Needs Stem Cells if You Can Dedifferentiate?

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**Regenerative mechanisms involving either stem cell differentiation or dedifferentiation of mature cells have been described in various systems, but the relationship between such disparate mechanisms is unclear. Two recent papers, one by Sandoval-Guzmán et al. (2013) and one by Tata et al. (2013), address fundamental questions about the universality of reparative regeneration and whether mammals share any properties seen in lower vertebrates.**

If the ability to regenerate complex tissues such as the heart or the limb has evolved once, why should it not be retained throughout evolutionary time? At a fundamental level, we assume that developmental and regenerative mechanisms are indeed universal. This expectation allows us to use zebrafish or chick embryos to understand human developmental disorders or reveal the mechanisms of limb regeneration in salamanders and cardiomyocyte regeneration in zebrafish for direct application to humans. Two recent papers, one by Sandoval-Guzmán et al. (2013) and one by Tata et al. (2013), explore the underlying regenerative processes in mice and salamanders and provide insight into shared and unique regenerative mechanisms in these two different systems. They approach the question of whether proliferative cells that provide the raw material for regeneration arise from a population of resident stem cells or from dedifferentiation of mature cells.

Significant advances have been made in understanding the cellular strategies used during reparative organ regeneration, which occurs with ease in salamanders such as axolotls and newts. Following amputation of the limb, for example, the epidermis rapidly heals the wound and underlying mesodermal cells accumulate to form a structure known as the blastema, which resembles a limb bud. The blastema proliferates and replaces the missing tissue by redifferentiation. The key to this type of reparative regeneration is the generation of proliferating mesodermal cells, which arise by a process of dedifferentiation whereby fully differentiated cells shed their characteristic genetic program of, for example, myosin synthesis or collagen synthesis and return to an undifferentiated, stem-cell-like state and begin to proliferate. This process is readily observable histologically and in muscle was well established by earlier electron microscopic observations (Hay, 1959) where multinucleate myofibers could be seen fragmenting into single cells and entering the blastema. The dedifferentiation, fragmentation, and proliferation of newt muscle fibers is also readily seen in vitro (Tanaka et al., 1999) and when fluorescently labeled newt myotubes are implanted into a blastema their progeny can be seen in regenerated tissues, demonstrating the

cell-intrinsic ability of such processes (Lo et al., 1993).

An entirely different strategy for reparative regeneration is employed by invertebrates such as planarians who maintain a large population of stem cells known as neoblasts throughout their bodies. These cells migrate to the site of damage and replace the lost tissues. Since the discovery of reservoirs of stem cells throughout the tissues of the mammalian body, it has been assumed that mammalian reparative regeneration would be promoted by the "multiply your stem cells" route (like planarians) rather than the "dedifferentiate, proliferate, redifferentiate" route (like axolotls and newts). The existence of these stem cell pools means that there is no need to be able to dedifferentiate since regeneration could presumably rely solely on stem cells.

But what of stem cells in axolotls and newts? Who needs stem cells if you can dedifferentiate? Recent studies in salamanders clearly demonstrated the presence of stem cells in the ependymal lining of the brain (Maden et al., 2013; Berg et al., 2010) or Pax7+ satellite cells in muscle (Morrison et al., 2006), but the expectation from such studies was that regenerated muscle would arise by dedifferentiation, fragmentation, and proliferation of myotubes and that satellite cells would play only a minor role. The paper by Sandoval-Guzmán et al. now explains the relevance of Pax7+ satellite cells to muscle regeneration during the regeneration of the limb and comes to a very surprising conclusion about species differences underlying regenerative mechanisms. The authors genetically labeled muscle fibers through electroporation of several vectors into the limb, including a muscle-specific Cre, which generated fluorescently labeled myonuclei while leaving the Pax7+ satellite cells, which reside outside the basement membrane of the fiber, unlabeled. When newts were used for experiments and limbs were amputated through the upper arm, fluorescently labeled nuclei could be seen along the entire length of the regenerated muscle, indicating that labeled mature cells had contributed to regenerating the new muscle through a dedifferentiation process. In striking contrast, when the same experiment was done in the axolotl, no labeled nuclei were seen in the regenerated muscle, indicating that instead new muscle fibers had arisen from unlabeled Pax7+ satellite cells.

The second paper, by Tata et al., concerns the epithelium of the mouse trachea, which contains several cell types including secretory cells, ciliated cells, neuroendocrine cells, and basal stem cells. This tracheal epithelium can readily regenerate after acute injury in which case proliferation of the stem cells is enhanced and redifferentiation replaces missing secretory and ciliated cells (Rock et al., 2009). Since there is a defined stem cell population, one would not expect to observe newt-like dedifferentiation in this system. However, when CK5-expressing basal stem cells are genetically ablated following inhalation delivery of doxyclycine, a 2-fold increase in the proliferation of secretory cells (marked by SCGB1A1) is observed. Under these circumstances, Tata et al. posed the question of whether one secretory cell could proliferate and directly make another secretory cell or whether dedifferentiation was taking place. When secretory cells were lineage labeled with YFP and the basal stem cells were ablated, Tata et al. observed some cells that were YFP labeled and morphologically indistinguishable from basal stem cells and that expressed several basal cell markers (including CK5), thus showing that they had dedifferentiated back to stem cells before generating new secretory and ciliated cells. In vitro experiments using spheres generated from pure secretory or basal cells showed the same result—spheres clonally derived from secretory cells contained a proportion of cells that expressed the CK5 stem cell marker. When clonally derived spheres from the two different cell types, secretory or basal, were cultured together in the same well, there was no inhibition of dedifferentiation from basal-cell-derived spheres, suggesting that direct cell contact (not a secreted factor) normally inhibits dedifferentiation in the multilayered tracheal epithelium in order to maintain the relative proportions of different cell types.

This mouse study reveals two unexpected newt-like behaviors. First, differentiated mammalian cells can dedifferentiate, become proliferative, and then redifferentiate into several cell types. Second, the proportionality of cell types within a tissue is homeostatically regulated by cell contact with the stem cell—a behavior also seen in the newt brain with regard to the maintenance of dopaminergic neurons (Berg et al., 2010). This is a striking example of universality of mechanisms and suggests a deep evolutionary origin for this cell behavior. But what of the apparent lack of universality between newts and axolotls, at least with regard to muscle regeneration? Perhaps it doesn't matter how you generate undifferentiated blastemal cells, and any source of proliferating cells will do. However, these findings implicate fundamental differences in regenerative mechanisms between two closely related species. Do we now have to go back and repeat newt experiments on axoltols and vice versa, and does this mean regeneration has evolved multiple times? Finally, if one salamander uses a mammalian strategy involving stem cells and another uses a dedifferentiative strategy, this immediately poses the question of whether other fundamental aspects of limb regeneration are also divergent, for example the neurotrophic control mechanisms. If so, then I believe that this is good news for the eventual goal of induction of mammalian regeneration, because there may be more than one way to achieve success.

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